IJMST

ISSN: 0974-5343
IJMST (2008), 1(1):1-12

Hepatitis C Virus (HCV) Genotypes: An Overview

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ABSTRACT :
This article presents a brief overview of hepatitis C virus (HCV) genotyping. HCV, an important causative agent of liver diseases, has six major genotypes and several subtypes. The genotypes and subtypes have been classified on the basis of their difference from each other in nucleotide sequence over the viral genome. These genotypes behave differently during course of infection and response to anti-viral treatment. This review describes a brief report on types and subtypes of HCV, origin of their diversity and the countrywise status of HCV genotypes. This also describes the impact of HCV genotypes on molecular pathology of HCV infection and their response to anti-viral therapy. All these informations are assumed to be helpful in planning future strategies for therapeutic management of HCV infection. To understand more about genetic diversity of HCV, there is a need to conduct further studies on various aspects of HCV-genotypes.

KEYWORDS : HCV, Genotype, subtypes, diversity, pathogenesis.

INTRODUCTION

The hepatitis C virus (HCV) is a positive-stranded RNA virus that was first characterized in 1989 by Choo et al. [1]. It was soon identified as the main causative agent of the previously called post transfusion non-A, non-B hepatitis.[2] HCV belongs to Flaviviridae family and its genome has 9.5-kb positive stranded RNA that encodes three structural proteins (core, envelope E1 and E2) and at least seven nonstructural proteins (NS1, NS2, NS3, NS4A, NS4B, NS5A and NS5B) [3,4]. The envelope proteins are localized on the outer surface of the virus and are the first contact with host cells during infection (Fig.1).

The E2 envelope protein is the main component of the two envelope proteins and assumed to be the primary mediator of virus attachment and cell entry [5]. Functions of viral envelope proteins are not only limited to virus binding and entry to the cells but they also play an important role in the regulation of host cell functions. E2 protein has been shown to provide co-stimulatory signals for T-cell activation [6] though its binding to NK cells inhibits their functions[7].

The genome of this virus is highly variable, and till date, six major HCV genotypes and almost 80 subtypes have been reported[8]. The existence of such evolutionary diverse forms of HCV is not only interesting from the point of view of viral phylogeny and taxonomy but, at the same time, it involves serious clinical, epidemiological and even forensic implications. Many studies demonstrated the association between HCV genotype and the responsiveness to antiviral combination therapy with pegylated interferon alpha and ribavirin[9], making HCV genotyping an indispensable tool for the tailoring of antiviral treatment and the diagnostic follow-up of the patients[10].

In view of several interesting findings related to HCV genotypes in last few years, it was considered worth to review and report important observations made on various aspects of HCV-genotyping. Present article gives a brief overview on types and subtypes, origin, global status and pathogenesis and therapeutic implications of HCV-genotypes.

HCV-genotypes and subtypes :

A recent international classification based on comparison of nucleotide sequences of HCV variants recovered from infected individuals and different geographical regions revealed the existence of at least six major genetic groups, termed as HCV genotypes. On average, these differ in 30-35% of nucleotide sites over the complete genome. Whereas variability is more prominent in E1 and E2 regions, sequence of core gene and non-structural regions, like NS3 often remains conserved.

ISSN: 0974-5343
IJMST (2008), 1(1):1-12

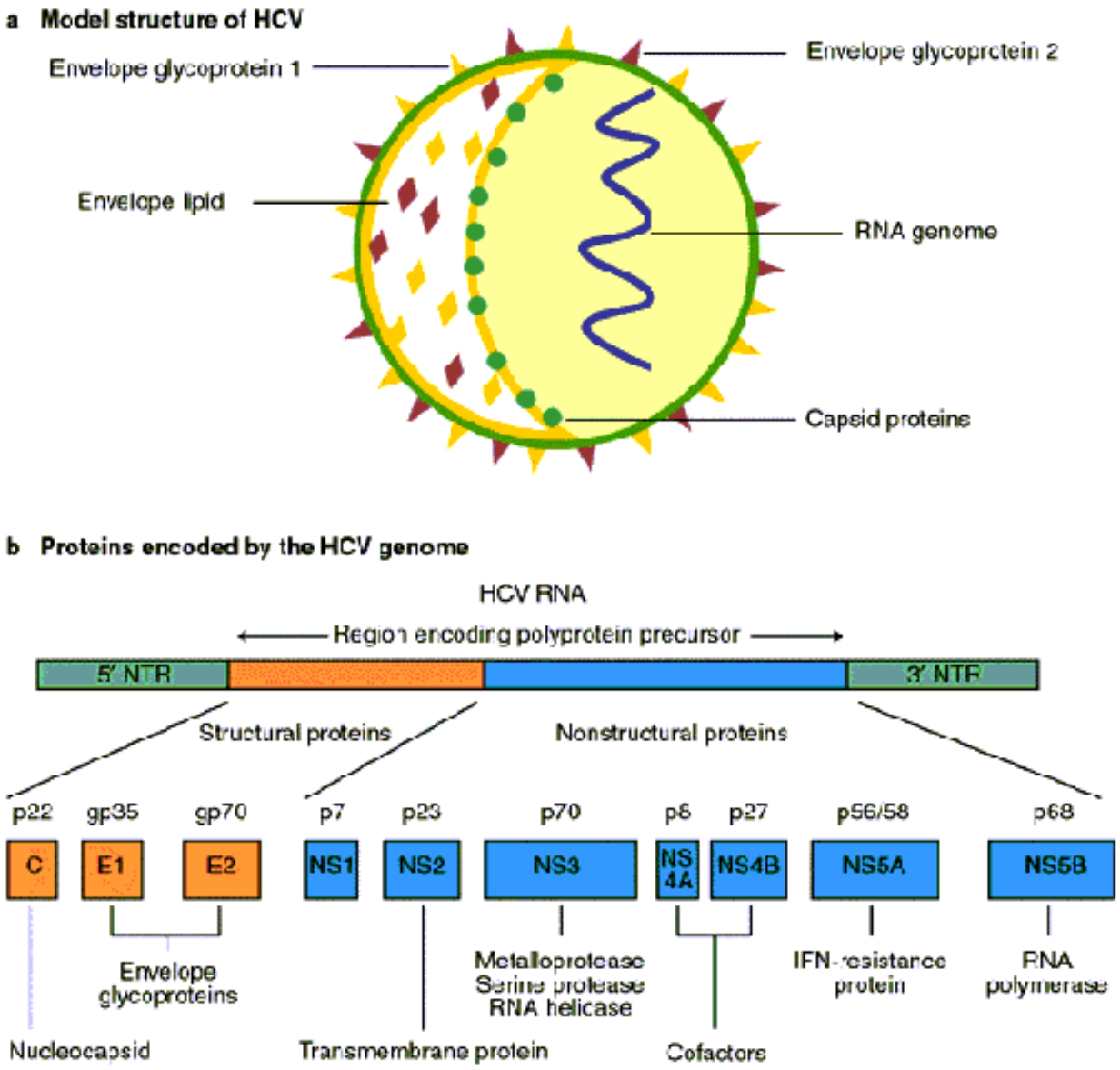

Fig.1a Model Structure of HCV Fig.1b Proteins encoded by the HCV Genome

The lowest sequence variability between genotypes is found in the 5`UTR, where specific sequences and RNA secondary structure are required for replication and translation function.

HCV genotypes have been further classified into more than 80 subtypes[8]. Each of the six major genetic groups of HCV contains a series of more closely related subtypes that typically differ from each other by 20–25 % in nucleotide sequences, compared with the >30 % divergence between genotypes[11]. Some, such as genotypes 1a, 1b and 3a, have become distributed very widely as a result of transmission through blood transfusion and needle-sharing between infecting drug users (IDUs) over the past 30–70 years and now represent the vast majority of infections in Western countries.

Despite the sequence diversity of HCV, all genotypes share an identical complement of collinear genes of similar or identical size. However, there is a marked variation in their capability to express a protein that is generated by a translational frameshift at codon 11 of the core gene[12-14]; both the frameshift site and potential size of this novel coding sequence are very poorly conserved between and within genotypes. This contrasts with the evolutionarily conserved nature of so many other aspects of

HCV replication and supports the idea that this 'gene' is more likely to be a computational artifact that has arisen from RNA structure-imposed constraints on third-codon position variability in the core gene[15].

Origin of genotyping:

Several studies were planned to relate genetic diversity to routes of HCV transmission. However, there was an absence of obvious transmission routes in those areas where greatest genetic diversity was seen [16-18]. In contrast to hepatitis B virus (HBV), there is currently no evidence that HCV or HCV-like variants infect Old World ape or monkey species[19]. Therefore, despite analogies with the introduction and spread of human immunodeficiency virus type 1 (HIV-1) and HIV-2 infections in humans through cross-species transmission of simian viruses from chimpanzees and mangabeys[20-21], it would be highly speculative and currently unjustified to imagine that HCV originated in these human populations as a result of similar cross-species transmission. On the other hand, it has been discovered that a very distantly related, HCV-like virus, GB virus B [22], infects tamarins and/or other New World primates. The existence of this homologue in such a distantly related primate species certainly allows for the possibility that HCV or HCV-like viruses may indeed be distributed more widely in primates than was thought previously.

It is difficult to estimate the length of time that HCV has been present in human populations. The diversity of variants within genotypes 1, 2 and 4 in sub-Saharan Africa and of genotypes 3 and 6 in South-East Asia suggests that HCV may have been endemic in these populations for considerably longer than in Western countries. The diversity of variants observed in west African genotype 2 sequences predicts a time of origin for this endemic pattern of infection of approximately 200–250 years ago, whilst different genotypes would have diverged from each other about 100 years earlier[23].

The major features of HCV structure, replication, transmission and ability to establish persistent infection are shared between all known variants. Indeed, viewed purely as a survival machine, the widespread distribution of genotypes 1–6 in human populations indicates that each is equally successful in maintaining infections in human populations. Despite this obvious evidence for phenotypic similarity, there is growing evidence for genotype-specific differences in persistence and interactions with innate cell defenses and the immune system.

The processes of neutral and adaptive evolution of HCV operate during the course of chronic infection within an individual. Sequence diversity is generated continually during virus replication, as RNA copying by the virally encoded RNA polymerase (NS5B) is error-prone and the replicating population is so large. Ongoing error rates between 1 in 10 000 and 1 in 100 000 bp copied, which are typically found for RNA polymerases[24-25], combined with a rate of virus production of up to 1012 virions per day[26], would produce a highly genetically diverse population of variants, containing mutants that differed at every nucleotide position and every combination of paired differences from the population mean or consensus.

The genotype epidemiology and natural history of infection with HCV favour recombination. A wide range of genotypes circulates in the main risk groups for HCV in Western countries, including 1a and 3a in Intravenous Drug Users (IDUs) and 1b, 2a–2c and 4a throughout the Mediterranean area. In these areas, infection is often characterized by multiple exposures around the time of primary infection, such as frequently repeated needle-sharing with several infected individuals over short time-intervals in the case of IDUs and the contamination of blood products, such as factor VIII clotting factor concentrates, with multiple HCV-positive plasma units. Indeed, even ongoing, chronic HCV infection does not protect from reinfection in experimentally challenged chimpanzees[27] or in HCV-contaminated blood or blood-product recipients, such as thalassaemics and haemophiliacs[28-30].

There is little experimental information on the potential viability of inter- or intra-genotype recombinants of HCV, although it has recently been observed that most combinations of genotype 1a and 1b sequences in the non-structural region of the genome fail to generate a viable replicon[31], implying the existence of incompatibilities between variants that show

IJMST

ISSN: 0974-5343
IJMST (2008), 1(1):1-12

TABLE 1 : Global status of HCV genotypes in HCV-RNA+ve populations

Country	Group	No. cases studied for genotypes (All HCV-RNA+)	Prevalence of genotypes Type	%prevalence	Reference
Pakistan	Normal population	48	G3	81%	35
			G1	9.5%	
			G2	2.4%	
South India	Renal transplant cases	258	G1b	43.4%	36
	Haemodialysis patients	46	G3b	30.2%	
			G3a	3.4%	
Brazil	Sickle cell anemia patients	41	G1b	63.0%	37
			G1a	21.0%	
			G3a	16.0%	
Northeastern Brazil	Haemodialysis patients	19	G1a	42.0%	38
			G3a	36.8%	
			G1b	21.0%	
Mexico	Haemodialysis patients	10	G1a	40.0%	39
			G1b	20.0%	
Central Italy	Normal population	448	G1	49.0%	40
			G1a	8.1%	
			G1b	37.0%	
			G3a	11.8%	
			G4	3.6%	
			Mixed	0.7%	
Spain	Liver diseases patients	108	G1b	86.1%	41
			G1a	7.4%	
			G3	3.7%	
			G4	1.9%	
			G2	0.9%	
	Random patients	413	G1b	69.5%	
			G1a	12.1%	
			G3	9.9%	
			G4	4.8%	
			G2	2.7%	
Brazil	Haemodialysis patients	163	G1a	75.0%	42
			G3a	16.7%	
			G1b	8.3%	

TABLE 1 : Global status of HCV genotypes in HCV-RNA+ve populations (contd.)

Country	Group	No. cases studied for genotypes (All HCV-RNA+)	Prevalence of genotypes Type %prevalence	Reference
Northeast Mexico	Hepatitis and cirrhosis patients	147	G1a 28.6% G1b 37.4% G1a/1b 4.1% G2a 1.4% G2b 8.8% G2c 0.7% G2a/2c 2.7% G3 2.0% G3a 10.2% G4 0.7% G4c 0.7%	43
China	Vountary donors	13620	G1a 18.0% G2a 23.0% G3a 41.0% G3b 18.0%	44

approximately 20 % sequence divergence. Despite these in vitro observations, recombinant forms of HCV have been observed in nature, including a variant formed from structural genes of genotype 2 and non-structural genes from genotype 1b that was found in infected IDUs in St Petersburg, Russia [32-33], and a possible 1a/1b recombinant in Peru [34].

Global status of genotypes :

Although genotypes 1, 2 and 3 are responsible for more than 90% of the infections in North and South America, Europe and Japan [23], prevalence and distribution peculiarities of HCV genotypes are linked to geographical location and mode of transmission [23]. Three broad patterns of genotype distribution have been identified to date [11]. One pattern, characterized by high genetic diversity, involves geographically discrete areas where HCV has been endemic for a long time such as West Africa with types 1 and 2, Central Africa with type 4, and Asia with types 3 and 6. Another pattern involves areas with a few subtypes circulating in specific risk groups, e.g. subtype 3a in drug addicts. The third pattern involves areas where a single subtype is present, such as in Egypt with subtype 4a and South Africa with subtype 5a. Table 1 indicates the prevalence of different HCV genotypes studied in normal and patient populations from several countries.

A different pattern of sequence diversity is observed in parts of Africa and South-East Asia. Here, there are close associations between genotypes and specific geographical regions. Infections in western Africa are caused predominantly by HCV genotype 2 [45-49], whereas those in central Africa, such as the Democratic Republic of Congo and Gabon, are caused by genotypes 1 and 4 [50-55]. In both regions, there is a remarkable diversity of subtypes; for example, 20 of 23 HCV-seropositive blood donors in Ghana (western Africa) were infected by genotype 2, but each corresponded to a different and previously undescribed subtype [49]. This diversity is reproduced in Guinea, Benin and Burkina Faso (central/western Africa), where 18 different subtypes of genotypes 1 and 2 were found in samples from 41 HCV-infected individuals [47]. These field observations reflect both the huge genetic diversity of genotypes 1, 2 and 4 and, also, its probable long-term presence in human populations in these parts of Africa. Taking this geographical mapping further, genotypes 3 and 6 show similar genetic diversity in southern and eastern Asia [56-58].

The model that is suggested by these genotype distributions is that HCV has been endemic in sub-Saharan Africa and South-East Asia for a considerable time, and that the occurrence of infection in Western and other non-tropical countries represents a relatively recent emergence of infection in new risk groups for infection [59]. In the 20th century, parenteral exposure to blood-borne viruses became frequent through the widespread adoption of blood transfusion. The use of unsterilized needles for injections and vaccinations and, most specifically, to industrialized countries, injecting drug using and the sharing of injection equipment is the major cause of particular exercise. These new routes for transmission account for the epidemiological and genetic evidence for recent epidemic spread of HCV over the past 50 years in Europe, Egypt and elsewhere [59, 60-62].

Pathogenesis and response of genotypes to treatment:

In common with other RNA and DNA viruses, HCV has developed a range of cell-defense evasion mechanisms that are centred around the activities of NS5A [63-65]. Whilst NS5A is a necessary part of the virus replication complex, it shows additional activities in binding to and inactivating PKR [66], blocking apoptotic pathways through sequestration of p53, modulation of intracellular calcium levels and binding to growth factor receptor-bound protein 2 [67-68]; and induction of anti-inflammatory interleukin 8 secretion [69]. It has also recently been shown that the HCV NS3/4A protease blocks the phosphorylation and signalling function of the antiviral IFN regulatory factor 3 [70]. The E2 protein, when expressed as a non-glycosylated, cytosolic protein [71], also appears to bind to and inhibit PKR as a result of sequence similarity to the phosphorylation domains of PKR and to e1F2 [72]. Interestingly, the degree of similarity to this 'homology' domain was greatest for genotype 1 variants and it was proposed that this contributed to the greater resistance of this genotype to IFN therapy.

One possible explanation for the differences in the outcome of infection between variants and genotypes of HCV is that they interact differently with host cells and achieve varying degrees of effectiveness in counteracting cell defences. Most obviously, the greater similarity of the E2 protein of genotype 1 to the phosphorylation domains of PKR and e1F2 has been suggested to explain its greater clinical resistance to treatment. However, further studies have generally not confirmed this hypothesis, with little correlation between the E2 sequence and response between genotypes or subtypes 1b, 2a, 2b, 2c, 3a and 4c/d (4a) [73-75].

Beside epidemiological considerations, HCV genotyping also has a clinical impact. Treatment for chronic hepatitis C includes standard or pegylated (PEG) interferon-α (IFN-α) in combination with ribavirin [76-77]. HCV genotype is a strong independent predictor of sustained virological response, which is defined by an undetectable HCV RNA level 24 wk after the end of treatment [78]. Genotypes 2 and 3 are significantly associated with a better therapeutic response, and knowledge on the HCV genotype is now used for tailoring therapeutic modalities.

More promising evidence for a relationship between virus sequence and persistence/treatment resistance was demonstrated in the region of NS5A that interacts with PKR. Long before its function was known, it was observed that there was a clustering of amino acid changes in NS5A during IFN treatment. An association was also found between treatment response and possession of the so-called 'prototype' NS5A sequence in the region where mutations occurred [79]. Prototype 'IFN-sensitivity determining region' (ISDR) sequences were also associated with higher circulating virus loads in untreated patients [80]. As the ISDR colocalizes with the part of NS5A that interacts with PKR, it was suggested that PKR evasion was a key determinant in the persistence of HCV and, potentially, other aspects of virus–host interaction.

Since the original study, several groups have sought to reproduce the findings of a dependence on ISDR sequence of treatment response in other patient cohorts. Despite highly variable results between studies, a recent meta-analysis of all the available data has demonstrated a clear correlation between the prototype ISDR sequence and treatment resistance and, as a corollary, a large number of diverse amino acid changes in non-responders [81]. It has also been shown that the same differential response exists in HCV genotype 2a and 2b infections [82]. In trying to unravel the mechanism of this interaction, it remains curious that while the 'prototype' ISDR sequence of NS5A is found specifically in individuals who resist IFN therapy, there is no evident selection

for this sequence in viruses with non-'prototype' sequences that are treatment-sensitive.

One theory is that the sequence in NS5A is under strong immune selection and shows varying degrees of freedom to mutate towards the most biologically active sequence for each genotype. NS5A is indeed known to contain a high concentration of T- and B-cell epitopes [83-85] and it is possible that immune selection in many individuals drives the ISDR or neighbouring sequence away from the prototype in individuals with certain HLA types that target epitopes in this region. A poorly functioning NS5A protein may make the infecting virus more sensitive to intracellular antiviral responses and, thus, to a greater likelihood of spontaneous viral clearance, as well as increased susceptibility to IFN therapy in those who remain viraemic. Similar immune-mediated selection may underlie the observation of treatment-induced amino acid changes in other functional regions of NS5A, such as V3 and a second region at positions 2282–2302 [86-88].

Infections by HCV genotypes 2 and 3 are generally much more responsive to IFN treatment may be because a far greater proportion of individuals recognize the prototype NS5A protein immunologically. Subsequent evolution of the infecting virus with a functionally impaired NS5A protein makes it less able to resist the further assault of exogenously administered IFN used for therapy. Human population-specific differences in the frequencies of HLA types in different study groups may also go some way to explaining why the association of 'prototype' ISDR (and potentially sequences in other NS5A regions) with treatment resistance varies so much between studies in Japan and Europe [83].

There is a clear difference between genotypes in their susceptibility to treatment with IFN monotherapy or IFN/ribavirin (RBV) combination therapy. Typically, only 40–50 % of individuals infected chronically with genotype 1 HCV on monotherapy and combination therapy, respectively, exhibit complete and permanent clearance of virus infection. This long-term response rate is much lower than the rates of 50 and 70–80 % that are observed on treatment of HCV genotype 2 or 3 infections.[89-90] This difference has proved to be highly significant in patient management and has led to the use of higher doses and longer durations of treatment for type 1 (and type 4) infections, in order to achieve acceptable efficacy. In numerous multivariate analyses, genotype-specific differences in treatment response have been shown to be independent of host variables, such as stage of disease progression, age, duration of infection, sex and HIV and other virus co-infections. It is similarly independent of virus-specific factors, such as pre-treatment viral load, although this also correlates independently with response.

CONCLUSION :

The current report presents a concise and brief update on important aspects of HCV-genotypes. There are six major genotypes of HCV which have been further classified into several subtypes termed as Isotypes or Quasispecies. Whereas six genotypes differ by 30-35% from each other in nucleotide sequences over the complete viral genome, subtypes differ by mere 20-25%. It is difficult to ascribe genetic diversity to a particular cause as several factors are involved in diversification. Based on evaluation of their presence globally, there is a wide variation in the overall prevalence of HCV genotypes in different countries. HCV genotypes have different impact on pathogenicity of infection and they also respond differently to the anti-viral treatment given to HCV infected patients. To understand more about genetic diversity of HCV, there is a need to conduct more studies to resolve the problem of its origin, impact on ensued diseases and also to work out a strategy to treat patients infected with different HCV-genotypes.

ACKNOWLEDGEMENT :

The authors thank and appreciate the financial aid provided by ICMR, New Delhi, India to conduct this study. Authors are also thankful to Mrs. Suman Rawat for preparing this manuscript.

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